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(54) Title: PEST CONTROLLING AGENTS

(57) Abstract

A method of controlling a pest which comprises bringing into contact with the pest a pest-controlling amount of an agent selected from the group consisting of (1) a non-formamidine -p-octopamine agonist having substantial activity toward an octopamine receptor present in the pest; (2) a compound directly and substantially stimulating the enzyme, adenylate cyclase; and (3) a cyclic adenosine monophosphate (cAMP) analogue.

DESCRIPTION

PEST CONTROLLING AGENTS

Technical Field

The present invention relates to pest controlling agents having pest controlling activity and to methods of controlling pests by treatment with the aforementioned agents.

Background Art

Despite the recent development and great promise of such advanced pest controlling compositions as chemical sterilants, pheromones or ecologically-based insect control strategies, it is doubtless that, at present, the use of chemical pesticides still plays a predominant role. The use of insecticides often represents the difference between profitable crop production for farmers and no marketable crop at all, and the value of insecticides in controlling human and animal diseases has been dramatic.

Therefore, in parallel to the aforementioned newer technologies for pest control, there has been active research and investigation into the detailed biochemical modes of action of existing known chemical pesticides. Thus, for example, Nathanson et al., Molecular Pharmacology 20:68-75 (1981) presented evidence indicating that the formamidine pesticides chlordimeform (CDM) and N-demethylchlordimeform (DCDM)

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may affect octopaminergic neurotransmission. CDM and
DCDM have been reported to mimic the effects of octo-
pamine in stimulating light emission in the firefly
lantern (Hollingworth, R. M. et al., Science, 208:
74-76 (1980)), and in effecting nerve-evoked muscle
responses in the locust leg (Evans, P.D., Nature,
287:60-62 (1980)). Nathanson et al., supra, found
that DCDM, which is the probable in vivo metabolite of
CDM, is about six-fold more potent than octopamine
itself as a partial agonist of light organ octopamine-
stimulated adenylate cyclase. Stimulation by the
formamidines resulted in increased formation of the
intracellular messenger, cyclic AMP. This stimulation
was blocked by cyproheptadine, clozapine, fluphenazine
and phentolamine compounds, also known to block the
octopamine receptor. Nathanson et al. concluded that
DCDM is the most potent octopaminergic compound
described.

Similar results were observed by Hollingworth et
al. (reported in the Scientific Papers of the Insti-
tute of Organic and Physical Chemistry of Wroclaw
Technical University, No. 22, Conference 7 (1980)).
These authors demonstrated that certain formamidines
act on octopamine receptors to induce the synthesis of
cyclic AMP, and that this response is blocked by both
phentolamine and cyproheptadine, which are known to
act as octopaminergic antagonists in insects. The
authors also suggested that these formamidines are
potent stimulators of the octopamine sensitive adeny-
late cyclases in the thoracic ganglia of Periplaneta
americana, and in the ventral nerve cord and fat body
of M. sexta. The authors suggest that the stimulation

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of octopamine receptors underlies a number of toxic responses seen with formamidines on insects.

It should be noted that the presence of an insect adenylate cyclase enzyme which is sensitive to naturally occurring D(-) octopamine as a "neuro transmitter" has been known for some time (Nathanson et al., Science, 180: 308-310 (1973) (cockroach); Nathanson, ibid: 203: 65-68 (1979) (firefly); Evans, J., Neurochem, 30: 1015-1022 (1978) (cockroach)).

The study of cyclic AMP (cAMP) as a "second messenger" has led to the accepted model that a hormone or neurotransmitter binds at a cell-membrane bound receptor, which activates adenylate cyclase to a form capable of converting ATP in the cytoplasm of the cell into cAMP. cAMP then relays the signal brought by the hormone or neurotransmitter from the membrane to the interior of the cell. Agonists of the hormone or neurotransmitter are, by definition, capable of eliciting the same response (see, for example, Nathanson and Greengard, Scientific American, 237:108-119 (1977)). Once formed inside the cell, cyclic AMP presumably binds to a protein kinase which is then capable of phosphorylating appropriate proteins, etc.

Given the continuous need for increased selectivity and effectiveness in pest control agents, it became desirable that the greater understanding of the biochemical mode of action of the formamidines be utilizable in some manner to lead to a general rational formulation of pest control agents.

DISCLOSURE OF THE INVENTION

The present invention arose out of the initial observations by the inventor and others that the mode
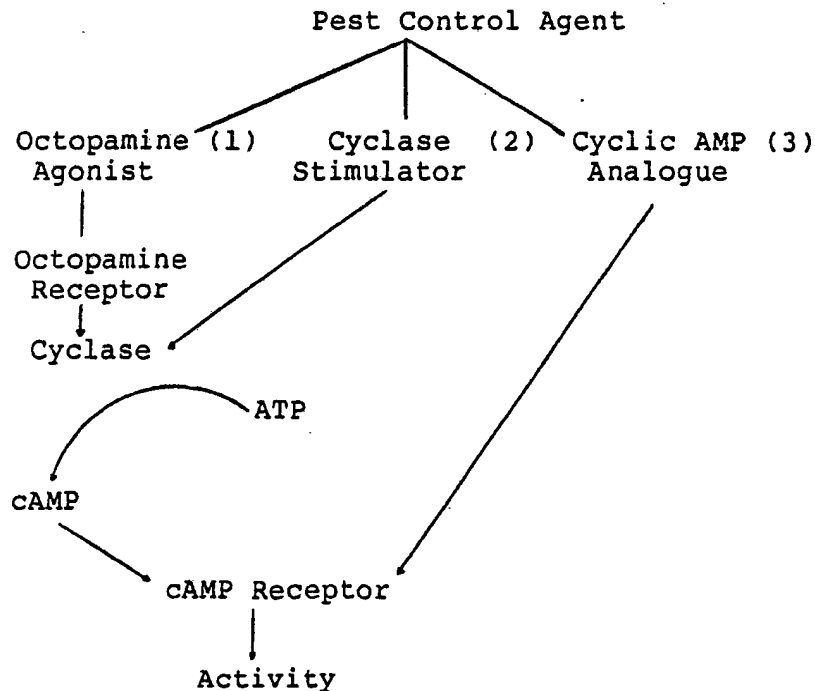
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of action of certain formamidine pesticides was
through their octopaminergic agonist activity on octo-
pamine receptors present in the pest, and that these
pest control agents were acting through generation of
cAMP as a "second messenger." It was not clear at the
time of these observations whether these were gene-
ralizable observations or were isolated instances. The
inventor has now observed that other, non-formamidine
octopaminergic agonists are also pest control agents.
In fact, the inventor's observations have extended
beyond the octopaminergic agonists to compounds that
directly effect adenylate cyclase and, further, to
cAMP analogues. Thus, in one embodiment, the present
invention provides a pest controlling composition
which comprises:

a compound having pest-controlling activity
towards said pest, selected from the group
consisting of

(1) a non-formamidine octopamine agonist
toward an octopamine receptor present in
said pest;

(2) a compound directly stimulating the
enzyme adenylate cyclase; and

(3) a cyclic adenosine monophosphate (cAMP)
analogue.

Scheme I illustrates the mode of action of the
agents of the invention:

Pest Control Agent

Octopamine (1)   Cyclase   (2)   Cyclic AMP (3)
  Agonist   Stimulator   Analogue

Octopamine
Receptor

Cyclase

ATP

cAMP

cAMP Receptor

Activity

Scheme I

Scheme I indicates the three types of pest control
agents having pest control activity useful in this
invention. These are either non formamidine octopa-
mine agonists (1), direct adenylate cyclase enzyme
stimulators (2), or cyclic AMP analogues (3). Octopa-
mine agonists (1) act by binding to a receptor which
activates adenylate cyclase which, in turn, produces
secondary messenger cyclic AMP. Enzyme stimulators (2)
also act through the production of cyclic AMP, but do
so by interacting directly with adenylate cyclase,

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bypassing the receptor. Once octopamine agonists or enzyme stimulators lead to the production of cyclic AMP, the cyclic AMP can bind to a cyclic AMP receptor generating hormonal-type activity. The third type of pest control compound, the cyclic AMP analogue (3), can bind to a cyclic AMP receptor generating hormonal-like activity. Compounds (1), especially, are highly selective pest control agents since vertebrate species--as opposed to invertebrate, e.g., insect, species--lack octopamine receptors.

In another embodiment of the invention, there is provided a method for controlling pests by treating said pests with an agent as hereinabove in an amount effective to provide pest control, by either pesticidal or pestistatic activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that three compounds, octopamine, NC7 (a clonidine) and di-demethylchlordimeform (DDCDM), are potent activators of adenylate cyclase in the nerve cord of the tobacco hornworm.

Figure 2 shows the effect of forskolin in activating adenylate cyclase in the firefly lantern. K_a ratio =0.22; V_{max} = 100%.

Figure 3 shows the effect of forskolin in activating adenylate cyclase activity in broken cell preparations from the nerve cord of tobacco hornworm larvae. K_a ratio = 0.20; V_{max} = about 100%.

Figure 4 shows an _in vivo_ leaf test of a composition containing forskolin in the presence or absence of a phosphodiesterase inhibitor. IBMX.

Figure 5 shows an *in vivo* antifeeding activity test for the cyclic AMP analogue p-chlorophenylthio-cyclic AMP in the presence or absence of IBMX.

Figure 6 shows an *in vivo* antifeeding activity test for leaves treated with n-butylaminobenzylthio cyclic AMP in the presence or absence of IBMX.

Figure 7 shows the amount of cyclic AMP analogue butylbenzylthio cyclic AMP remaining in pest tissue in the presence or absence of IBMX.

BEST MODE FOR CARRYING OUT THE INVENTION

The terms "pest controlling" or "pest controlling activity," used throughout the specification and claims, are meant to include any pesticidal (killing) or pestistatic (preventing the host plant from being eaten, or inhibiting, maiming or generally interfering) activities of a composition against a given pest at any stage in its life cycle. Thus, these terms not only include killing, but also include such activities as the production of behavioral abnormalities (e.g., tremor, incoordination, hyperactivity, anorexia, leaf walk-off behavior) which interfere with such activities such as but not limited to eating, molting, hatching, mobility or plant attachment. The terms also include activities of chemisterilants which produce sterility in insects by preventing the production of ova or sperm, by causing death of sperm or ova, or by producing severe injury to the genetic material of sperm or ova, so that the larvae that are produced do not develop into mature progeny.

The terms also include repellants, which are substances that protect animals, plants or products from

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insect attack by making food or living conditions
unattractive or offensive. These may be poisonous,
mildly toxic, or non-poisonous.

The terms also include attractants, food lures,
sex pheromones, aggregation pheromones, and the like.
Any compound which has such "pest controlling acti-
vity" as defined and which is (1) a non-formamidine
octopamine agonist toward a an (-)-p-octopamine recep-
tor present in the pest, (2) a direct stimulator of
adenylate cyclase, or (3) a cyclic AMP analogue, is
included in the present invention.

The question of whether a given compound (1) is a
non-formamidine octopamine agonist can be readily
answered by measuring adenylate cyclase activity of
the octopamine-sensitive adenylate cyclase present in
broken cell preparations of the firefly light organ.
Generally, the broken cell preparations are prepared
according to the method described in a paper by
Nathanson et al. (Molecular Pharmacology 20:68-75
(1981), which is herein incorporated by reference.
Specimens of Photinus pyralis are prepared by opening
their tail sections, cleaning them, removing the light
organs, and homogenizing the cyclase-containing frac-
tion. Adenylate cyclase activity is measured in
appropriate buffer-containing ATP and the compound to
be tested. If necessary, the compounds (1) to be test-
ed are initially solubilized and appropriate solvent
controls are run in parallel. The enzyme reaction is
initiated by addition of ATP, stopped by heating, and
centrifuged. Cyclic AMP can be measured by any test
which indicates the presence thereof, preferably by
the protein binding assay of Brown et al. (Advances in

Cyclic Nucleotide Research 2:25-40 (1972)). Normally,
the solution mixture contains a phosphodiesterase
inhibitor such as theophylline, so as to provide
linear measurements with respect to time and enzyme
concentration. The determination of the constant K_a,
which is the concentration of agonist (l) necessary
for half-maximal activation of cyclase activity, is
carried out by measuring cyclase activity in the
preparation, and plotting the activity (above control
activity) versus the semilogarithm of the particular
agonist concentration. This is done for a series of
increasing concentrations until maximal activity
(Vmax) is reached. $K_a^{(1)}$ is then calculated from the
graph as the agonist concentration required for
one-half of Vmax. $K_a^{(1)}$ is compared with the constant
(K_a^{oct}) determined in an analogous way using $\pm$ p-octo-
pamine as the agonist. The ratio $K_a^{oct}/K_a^{(1)}$ is then
an indication of whether the compound (l) is better
(ratio greater than l) or worse (ratio smaller than l)
than ($\pm$)-p-octopamine. Maximal activation of enzyme
activity as a percentage of maximal activation seen in
the presence of ($\pm$) p-octopamine can be denoted as %
Vmax. (Although the naturally occurring insect neuro-
transmitter is (-)-p-octopamine, the definition of an
"agonist" in the present application is based on
($\pm$)-p-octopamine as the standard. Since an "agonist"
is a compound that mimics the effect of (-)-p-octopa-
mine, (-)p-octopamine itself, free of substantial
amounts of the (+) enantioner is not included in the
definition).

Generally, an octopamine agonist having a
$K_a^{oct}/K_a^{(1)}$ ratio greater than 0.05, preferably 0.05

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to 1000, most preferably 0.1 to 1000, as measured by
the firefly lantern test, is used. Also, generally,
octopamine agonists having Vmax anywhere between 5 and
upwards of 100%, preferably between 10 and upwards of
100%, of the Vmax of (+)-p-octopamine can be used. The
values of Vmax for any desired octopamine agonist are
not as important as the values of the ratio of K's. As
long as the $K_a^{oct}/K_a^{(1)}$ ratio falls within the stated
range, the Vmax values can vary widely.

In addition to the above method employing the
firefly light organ, octopamine-sensitive adenylate
cyclase can also be measured in tissue preparations
from the nerve cord of any desired particular insect
pest, using a modification of the method appearing in
Nathanson et al. (Science 180:308-310 (1973)) herein
incorporated by reference. In this modification
(which is not necessary if the firefly light organ is
used), dopamine (10 micromolar) and serotonin (10
micromolar) are added to all (including control) assay
tubes. This is done in order to be sure that the
tested compounds (1) are affecting only octopamine
receptors (known to be present in all insect nerve
cords) and not dopamine or serotonin receptors. Other-
wise, the procedure is identical to that described
above.

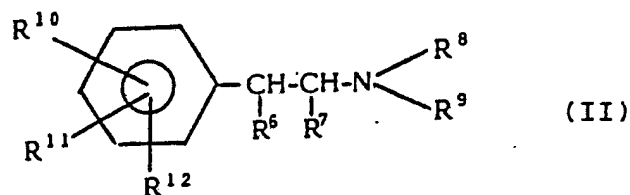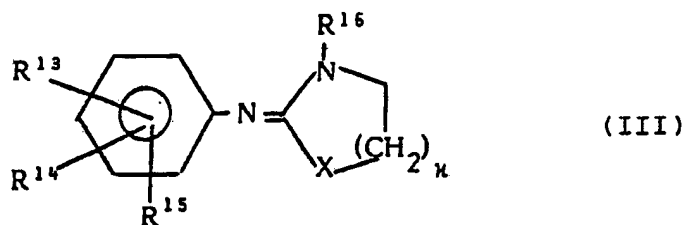By "formamidines" are meant compounds of formula
(I):
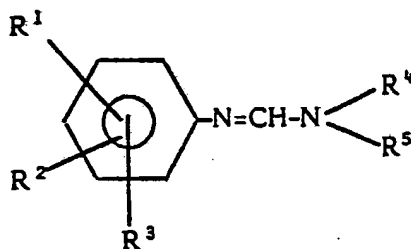
(I)

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R^4 and R^5 stand for hydrogen, lower alkyl or lower alkyl substituted by hydroxy or lower (C_1-C_6) alkoxy. R^1, R^2, R^3 are the same or different and selected from the group consisting of hydrogen, hydroxy, fluorine, chlorine, bromine, iodine, nitro, lower (C_1-C_6) alkyl, lower (C_1-C_6) alkoxy, lower haloalkyl, amino, mono lower alkyl amino, di-lower alkyl amino, hydroxy-substituted lower alkyl and lower acylamino.

Among the preferred non-formamidine octopamine agonists (1) are those belonging to the families of the phenylethylamines (II):

(II)

Cyclic Amidines (III):

(III)

where X is $N-R^{16}$, O, CH_2, or S, and n may be 1 or 2; such as 2-(phenylimino) imidazolidines (X=NH, n=1);

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2-(phenylimino) pyrrolidines (X= CH_2, n=1); 2-(phenyl-imino) oxazolidines (X=0, n=1); 2 (phenylimino) thiazolidines (X=S, n=1) and 2-(phenylimino) thiazines (X=S, n=2). See, e.g., the compounds in DeJong et al, Europ. J. Pharm. 69: 175-188 (1981);

2-benzylimidazolines (IV):

(IV)
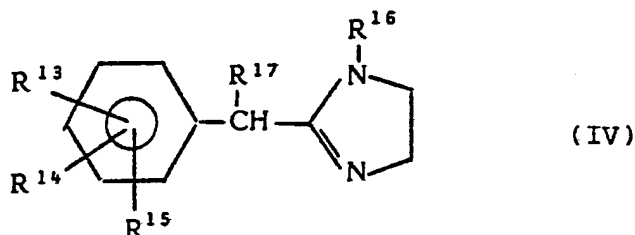
R^8, R^9, R^{16} stand for hydrogen, lower alkyl or lower alkyl substituted by hydroxy or lower (C_1-C_6) alkoxy; R^{17} is hydrogen or hydroxy. R^6 and R^7 are the same or different and selected from the group consisting of hydrogen, hydroxy and lower (C_1-C_6) alkyl. R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} are the same or different and selected from the group consisting of hydrogen, hydroxy, fluorine, chlorine, bromine, iodine, nitro, lower (C_1-C_6) alkyl, lower (C_1-C_6) alkoxy, lower halo-alkyl, amino, mono lower alkylamino, di-lower alkyl amino, hydroxy substituted lower alkyl and lower acylamino.

Also, in compounds of formulae (III) or (IV) above, R^{13}, R^{14} or R^{13}, R^{15} together may form a six membered phenyl, pyridine, diazine, or cyclohexyl ring fused to the noted phenyl ring. For example, systems
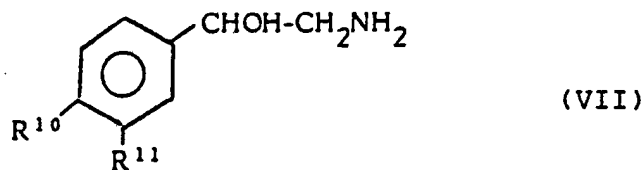
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of formulae V and VI can also be used:

(V)

(VI)
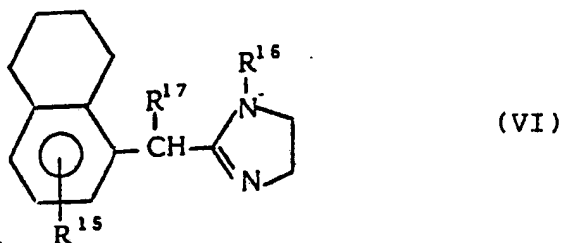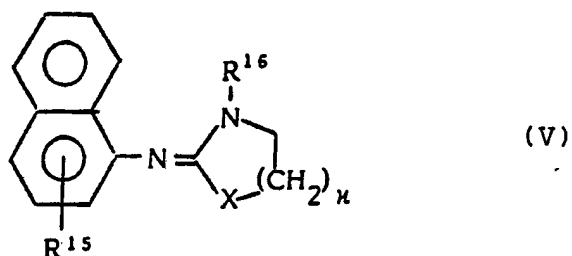
where R^{15}, R^{16}, R^{17}, and n are as defined previously.

Specific compounds useful as non-formamidine octopamine agonists (1) include phenylethylamines of the formula (VII):

(VII)

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where R^{10} is OH and R^{11} is CH_3, C_2H_5, i-C_3H_7, C_6H_{11}, NH_3, F, Cl, Br I, $NHSO_2CH_3$, OH, H or OCH_3; or where R^{11} is OH and R^{10} i-C_3H_7, CH_3, C_2H_5, C_6H_{11}, NH_3, Cl, Br, I, NH-SO_2CH_3, OCH_3 or H.

Other specific compounds (1) include cyclic amidines of the formula (VIII):

$$R - N = \left\langle \begin{array}{c} NH \\ NH \end{array} \right. \qquad \text{(VIII)}$$
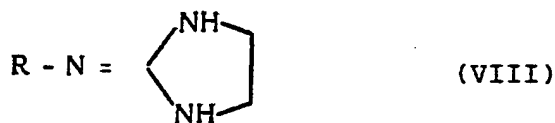
where R is phenyl; o-tolyl; 2,6 dimethylphenyl; 2,3 (cyclohexyl) phenyl; 2,6-diethylphenyl; 2,6-difluoro-phenyl; 2-chlorophenyl; 2,6-dichlorophenyl; 3-chloro-phenyl; 2,5-dichlorophenyl; 3,5-dichlorophenyl; 5-bromoquinoxaline; 2-methyl,3-bromophenyl; 2-chloro,4-methylphenyl; 3-fluoro,6-methylphenyl; 2-chloro,3-methylphenyl; 2,6-dichloro, 4-hydroxy-phenyl; 3,4-dihydroxyphenyl; or 4-chlorophenyl.
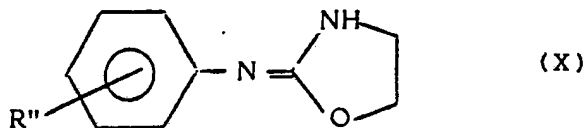
Other specific compounds (1) include cyclic amidines of the formula (IX):

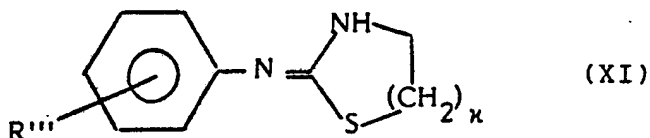$$R' - N = \left\langle \begin{array}{c} NH \\ \end{array} \right. \qquad \text{(IX)}$$

where R' is phenyl, o-tolyl, 2,6-dimethylphenyl, 2-chlorophenyl, 2,6-dichlorophenyl, 4-chlorophenyl, or 4-methoxyphenyl.

Other specific compounds (1) include cyclic amidines of the formula (X):

(X)

where R" is H, 2-CH$_3$, 2-6-diCH$_3$, 4-CH$_3$, 4-Cl or 2,6-diCl.

Other specific compounds (1) include cyclic amidines of the formula (XI):

(XI)

where R''' is 2,6-dimethyl; 2,6-dichloro; 2-6,diethyl; 2,4,6-trimethyl; 2,4 dichloro; 2,4 dimethyl; 2-chloro-4-methyl; 4-chloro-2-methyl; 4-chloro; 2-chloro; 2-methyl or 4-methyl;
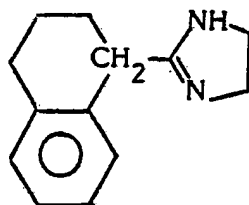
where n is 1 or 2.

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Other specific compounds (l) include 2-benzyl-imidazoline of the formula (XII):

$$R''''-CH_2 - \overset{NH}{\underset{N}{\diagdown}} \qquad (XII)$$
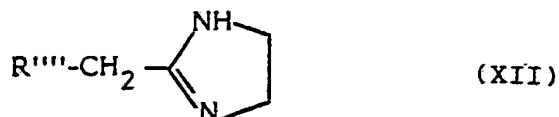
where R"' is phenyl, o-tolyl, 2,6-dichlorophenyl, 4-CH_3O phenyl, 2,3 naphthyl (naphazoline), 2,6-di-methyl, 4-^{t}Butylphenyl (xylometazoline), 2,6-dimethyl, 3-hydroxy, 4- Butylphenyl (oxymetazoline),

or

$$\overset{NH}{CH_2 - \underset{N}{\diagdown}}$$

(tetrahydrozoline)

The syntheses and preparation of phenylethylamines of formula (II) is described, for example, in LeClerc et al., J. Med. Chem. 23:738-744 (1980). The syntheses and preparation of cyclic amidines of formulas (III) or (IV) is described, for example, in Rouot, et al., Journal of Medicinal Chemistry, Vol. 19, 1049 (1976); Oxley et al, J. Chem Soc., 497 (1947), Faust et al., J. Org. Chem. 26: 4044 (1961); Van der Stelt

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et al., <u>Arzneim. Forsch</u> 15: 1251 (1965) or <u>Jen et al.</u>,
<u>J. Med. Chem</u> 15: 727 (1972) and <u>ibid</u>, <u>18</u>: 90 (1975).

Other examples of specific compounds (1) which are
octopamine agonists are listed in the Tables in the
accompanying examples.

The second type of pest controlling compounds (2)
useable in the present compositions are direct stimu-
lators of the pest enzyme adenylate cyclase. These
compounds bypass the receptor, and interact with one
or another of the associated catalytic or regulatory
subunits of adenylate cyclase, thereby stimulating the
formation of cyclic AMP. Suitable compounds can be
determined from assay of pest adenylate cyclase as
described above. Generally, a compound (at a concen-
tration of less than 1 millimolar) causing a stimula-
tion of adenylate cyclase of at least 10% that due to
a Vmax concentration of ($\pm$)-p-octopamine is preferred.
A direct stimulator of adenylate cyclase can be dis-
tinguished from an octopamine agonist in that the
stimulatory activity of the former (but not the
latter) at a concentration causing half-maximal acti-
vation of the enzyme, is not significantly reduced by
the addition of known octopamine receptor antagonists,
such as phentolamine or cyproheptadine, used at a
concentration of 100 micromolar.

Among preferred direct enzyme stimulators are
those belonging to the diterpenes, (XIII), forskolin
(XIV) and its derivatives:

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(XIII)
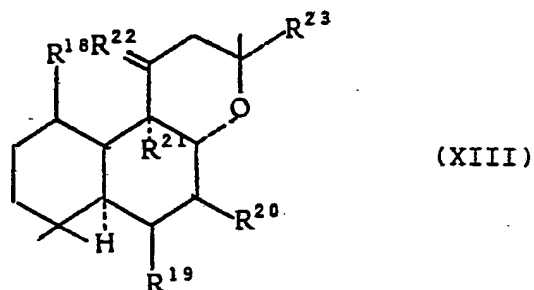
R^{18-23} stand for hydrogen, hydroxyl, oxy, keto, lower alkyl, lower alkene, lower alkoxy, carboxy and carboxyamino.

In forskolin, $R^{18}=R^{19}=R^{21}=OH$; $R^{20}=OCOMe$; $R^{22}=O$; and $R^{23}=CH=CH_2$.

Certain bacterial-derived toxins, such as cholera toxin can also be used as enzyme stimulators.

The preparation of forskolin derivatives is described in Seamon, K. and Daly, J. W., J. Med. Chem. 26: 436-439 (1983). The structure and action of cholera toxin are described in Van Heyningen, S., Biosci. Repts. 2:135-146 (1982).

The third type of pest controlling compounds (3) useable in the present invention are cyclic adenosine monophosphate analogues. These are compounds which have cyclic AMP activity, and are capable of binding to the appropriate pest protein kinase to activate the same. The potency of a particular cyclic AMP analogue can be determined from the calculated K_a and Vmax of the analogue for activating cyclic AMP-dependent protein kinase found in insect nerve cord or firefly lantern, using the method described by Nathanson in

Cyclic AMP: A Possible Role in Insect Nervous System
Function, Ph.D. Thesis, Yale Univ., 1973, pp. 81-82,
herein incorporated by reference. The K_a and Vmax for
the analogue can be compared, in the same tissue, with
the K_a and Vmax for cyclic AMP, itself, in stimulating
protein kinase.

Generally, a cyclic AMP analogue with a $K_a^{(3)}/$
K_a(cyclic AMP) ratio greater than 0.01, preferably
about 0.01 to 100 or more, most preferably 0.05 to 100
or more is used. Also, generally, cyclic AMP ana-
logues having a Vmax anywhere between 5 and upwards of
100% of the Vmax for cyclic AMP can be used.

A number of biologically active cyclic AMP ana-
logues have been synthesized. See Revankar and Robins,
in Handbook of Experimental Pharmacology, 58/I (ed. J.
Nathanson, J. Kebabian) pp. 17-151 (Spring-Verlag,
N.Y.) 1982. Among the preferred ones are 6-n-butyl-
amino-8-benzylthio-cyclic AMP; 8-p-chlorophenylthio-
cyclic AMP; 8-chloro-cyclic AMP; 8-bromo-cyclic AMP;
N^6-monobutyryl or N^6,2'-0-dibutyryl cyclic AMP;
7-deaza-cyclic AMP; and 1-deaza-cyclic AMP.

The molecular effects of compounds (1), (2) or (3)
in vitro correlate with the molecular effects in vivo.
However, it may be that a compound which is an excel-
lent agonist, stimulator or analogue in vitro does not
show good in vivo activity. Other factors, such as
possible metabolism, transport or absorption of the
compound may influence its overall effectiveness. One
of skill in the art, however, can by a simple pre-
liminary trial on the desired pest ascertain quite
quickly and routinely whether a chosen agent is useful
in vivo.

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In a preferred embodiment the agents (1), (2) or
(3) are used in combination with phosphodiesterase
(PDE) inhibitors, to yield synergistic pest control-
ling conpositions. The PDE inhibitors decrease a side
reaction which causes hydrolysis and/or breakdown of
the compound itself (in the case of cAMP analogues) or
of secondary messenger cAMP (in the case of octopamine
agonists or enzyme stimulators).

It should be noted that applicant has filed on
even date a commonly assigned patent application
entitled "Pest Controlling Compositions" having Serial
No. _____, which discloses compositions of octopa-
mine agonists (1), enzyme stimulators (2), or cAMP
analogues (3) with PDE inhibitors. The full disclo-
sure of this copending patent application is herein
incorporated by reference.

The pest controlling agents of the present inven-
tion can be formulated as dusts, water dispersions,
emulsions, and solutions. They may comprise accessory
agents such as dust carriers, solvents, emulsifiers,
wetting and dispersing agents, stickers, deodorants
and masking agents (see for example, Encyclopedia of
Chemical Technology, Vol. 13, page 416 et seq.).

Dusts generally will contain low concentration,
0.1-20%, of the compounds, although ground prepara-
tions may be used and diluted. Carriers commonly
include organic flours, sulfur, silicon oxides, lime,
gypsum, talc, pyrophyllite, bentonites, kaolins, atta-
pulgite, and volcanic ash. Selection of the carrier
can be made on the basis of compatibility with the
desired pest control composition (including pH,
moisture content, and stability), particle size,

abrasiveness, absorbability, density, wettability, and
cost. The agent of the invention alone or in com-
bination and diluent is made by a variety of simple
operations such as milling, solvent impregnations,
fusing and grinding. Particle sizes usually range
from 0.5-4.0 microns in diameter.

Wettable powders can be prepared by blending the
agents of the invention in high concentrations, usual-
ly from 15-95%, with a dust carrier such as bentonite
which wets and suspends properly in water. 1 to 2% of
a surface-active agent is usually added to improve the
wetting and suspendibility of the powder.

The pest-controlling agents can also be used in
granules, which are pelleted mixtures of the agents,
usually at 2.5-10%, and a dust carrier, e.g., adsorp-
tive clay, bentonite or diatomaceous earth, and
commonly within particle sizes of 250 to 590 microns.
Granules can be prepared by impregnations of the car-
rier with a solution or slurry of the agents and can
be used principally for mosquito larvae treatment or
soil applications. _

The agents can also be applied in the form of an
emulsion, which comprises a solution of the agents in
water immiscible organic solvents, commonly at 15-50%,
with a few percent of surface active agent to promote
emulsification, wetting, and spreading. The choice of
solvent is predicated upon solubility, safety to
plants and animals, volatility, flammability, com-
patibility, odor and cost. The most commonly used
solvents are kerosene, xylenes, and related petroleum
factions, methylisobutylketone and amyl acetate. Water
emulsion sprays from such emulsive concentrates can be

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used for plant protection and for household insect control.

The agents can also be mixed with baits, usually comprising 1-5% of agents with a carrier especially attractive to insects. Carriers include sugar for house flies, protein hydrolysate for fruit flies, bran for grasshoppers, and honey, chocolate or peanut butter for ants.

The agents can be included in slow release formulations which incorporate non-persistent compounds, insect growth regulators and sex pheromones in a variety of granular microencapsulated and hollow fiber preparations.

The pest controlling agents of the present invention will be applied depending on the properties of the particular pest controlling compound, the habits of the pest to be controlled and the site of the application to be made. It can be applied by spraying, dusting or fumigation.

Doses of the weight of the ingredients may typically vary between 0.001 - 100 lbs/acre, preferably between 0.001 - 5 lbs/acre.

Sprays are the most common means of application and generally will involve the use of water as the principal carrier, although volatile oils can also be used. The pest-control agents of the invention can be used in dilute sprays (e.g., 0.001-10%) or in concentrate sprays in which the composition is contained at 10-98%, and the amount of carrier to be applied is quite reduced. The use of concentrate and ultra low volume sprays will allow the use of atomizing nozzles producing droplets of 30 to 80 microns in diameter. Spraying can be carried out by airplane or helicopter.

Aerosols can also be used to apply the pest con-
trolling agents. These are particularly preferred as
space sprays for application to enclosures, particu-
larly against flying insects. Aerosols are applied by
atomizing amounts of liquified gas dispersion or bomb
but can be generated on a larger scale by rotary
atomizers or twin fluid atomizers.

A simple means of pest control agent dispersal is
by dusting. The pest controlling agent is applied by
introducing a finely divided carrier with particles
typically of 0.5-3 microns in diameter into a moving
air stream.

Any octopamine-receptor containing pest is treat-
able by the formulation of the present invention.
These pests include all invertebrate pests, including,
but not limited to, round worms (e.g., hookworm,
trichina, ascaris); flatworms (e.g., liver flukes and
tapeworms); jointed worms (e.g., leeches); molluscs
(e.g., parasitic snails); and arthropods (insects,
spiders, centipedes, millipedes, crustaceans (e.g.,
barnacles)).   In particular, included among the
arthropods are ticks; mites (both plant and animal);
lepidoptera (butterflies and moths and their larvae);
hemiptera (bugs); homoptera (aphids, scales); and
coleoptera (beetles). Also included are spiders;
anoplura (lice); diptera (flies and mosquitoes);
trichoptera; orthoptera (e.g., roaches); odonta;
thysanura (e.g., silverfish); collembola (e.g.,
fleas); dermaptera (earwigs); isoptera (termites);
ephemerids (mayflies)); plecoptera; mallophaga (biting
lice); thysanoptera; and siphonaptera (fleas); dic-
tyoptera (roaches); psocoptera (e.g., booklice); and

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certain hymenoptera (e.g., those whose larva feed on leaves).

Examples

Having now generally described this invention, the same will become better understood by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

IN VITRO METHODS

I. Determination of Octopamine Agonist Activity

A. Firefly Light Organ

Specimens of Photinus pyralis were collected in summer, frozen on dry ice, and stored at -90°. Under these conditions, octopamine sensitive enzyme activity remains stable for six months or longer. For each experiment, a number of insects were thawed and maintained at 4°C. Tail sections were opened through a dorsal midline incision and the abdominal cavity was cleaned out of all gut, fat reproductive organs, and ganglia. The light organs were then removed from the ventral cuticle, cleaned of any adhering nonlantern tissue, and homogenized (10 mg/ml) in 6 mM Tris-maleate buffer (pH 7.4). To prepare a P_2 fraction, the homogenate was diluted to a volume of 30 ml in 6 mM Tris-maleate and centrifuged at 120,000 x g for 20 minutes. The supernatant was discarded, and the pellet was resuspended by homogenization in 30 ml of buffer and again centrifuged at 120,000 x g for 20 minutes. The resulting pellet (P_2 fraction) was resuspended in a volume of 6 mM Tris-maleate equivalent to the starting amount and maintained at 0° until

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it was used. Alternatively, the homogenate may be used directly without preparing P_2 fraction.

Adenylate cyclase activation by test compounds was measured in test tubes containing (in 0.3 ml) 80 mM Tris-maleate, pH 7.4; 10 mM theophylline; 8 mM $MgCl_2$; 0.1 mM GTP; 0.5 mM ethylene glycol bis (beta-amino-ethyl ether)-N,N,N'N'-tetraacetic acid; 2 mM ATP; 0.06 ml of P_2 fraction; and the various compounds to be tested. Prior experiments had determined that, under these conditions, octopamine-sensitive adenylate cyclase activity is optimized. Test compounds were initially solubilized (prior to aqueous dilution) in water or (if soluble) in 50% (v/v) methanol. If insoluble in 50% methanol, compounds may be dissolved initially in 100% methanol, or 100% dimethylsulfoxide, or 100% polyethylene glycol. Final solvent concentration after dilution can be kept as high as 15% for methanol and DSMO and as high as 20% for polyethylene glycol. Appropriate solvent controls were run in parallel. The enzyme reaction (5 minutes at 30°) was initiated by addition of ATP, stopped by heating to 90° for 2 minutes, and then centrifuged at 1000 x g for 15 minutes to remove insoluble material. Cyclic AMP in the supernatant was measured by protein-binding assay, according to the method of _Brown et al._, _Adv. Cyclic Nucleotide Res._ 2: 25-40 (1970). Under the above assay conditions, enzyme activity is linear with respect to time and enzyme concentration, and phospho-diesterase activity is nearly completely inhibited. Previous experiments had shown that the cyclic AMP produced in this reaction co-chromatographs on Dowex AG-50X® resin with authentic cyclic AMP. Protein concentration was determined by the method of _Lowry et_

al., Journal of Biological Chemistry 193: 265-275
(1951).

B. Other Pest Tissues

In those cases in which other pest tissues are
used to measure octopamine agonist activity, the
procedure is identical to that above, except that the
tissue to be used is the brain, segmental ganglia, or
the entire nerve cord of the insect pest, with or
without the brain. The tissue is homogenized (usually
15mg/ml) as above in 6 mM Tris maleate, pH 7.4. Assay
conditions are identical to those described above
except that dopamine (10 micromolar) and serotonin (10
micromolar) are added to all (including control) assay
tubes when testing compounds which may affect recep-
tors other than octopamine receptors. This is done to
cancel out the effects of dopamine and serotonin
receptors which are usually present in nerve cord. It
assures that the compound tested is affecting only
octopamine receptors (known to be present in all
insect nerve cords).

II. Determination of Adenylate Cyclase Stimulating Activity

As detailed previously, the adenylate cyclase
assay, either in the firefly or in other insect pest,
is also used to identify a direct stimulator of adeny-
late cyclase.

III. Determination of Cyclic AMP-Dependent Protein Kinase Activity (for Determination of Activity of Cyclic AMP Analogues)

A P_2 pellet is prepared as described above from
either firefly lantern or insect pest nerve tissue. In

this assay, the pellet is used both as a source of protein kinase and as the substrate which is phosphorylated. The assay mixture (total volume 0.2 ml) contains: 10 micromoles sodium glycerol phosphate buffer, pH 7.4; 1 millimicromole gamma-^{32}P-ATP, approx. 10^6 cpm; 2 micromoles $MgCl_2$; 2 micromoles NaF; 0.4 micromoles theophylline; 0.06 micromoles EGTA; 10-100 micrograms protein of P_2 pellet; $\pm$ various concentrations of cyclic AMP or the cyclic AMP analogue to be tested (typically to give a final concentration of from $10^{-9} - 10^{-4}$M).

The reaction is initiated by the addition of tissue and the incubation is for 5 minutes at 30°C. The reaction is terminated by the addition of 4 ml of 7.5% trichloroacetic acid (TCA). 0.2 ml of 0.63% bovine serum albumin is added, the mixture is centrifuged at low speed, and the supernatant is discarded. The precipitate is dissolved in 0.1 ml of 1N NaOH and the TCA precipitation repeated 4 more times. The protein-bound ^{32}P is then redissolved in NaOH and counted in a scintillation spectrometer. The amount of increase in cpm over control tubes incubated in the absence of cyclic AMP or cyclic AMP analogue represents cyclic-AMP-dependent protein kinase activity. Activity constants are calculated as described previously.

IN VIVO METHODS

To test the pesticidal and pestistatic effects of some of the disclosed compounds, the effects on the feeding behavior of tobacco hornworms (_Manducca_ _sexta_) were investigated. This species is one of the several types of insects particularly susceptible to octopamine type insecticides. The ease of rearing this

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species from eggs in the laboratory and the ability to
maintain them on artificial media, makes it possible
to test compounds on large numbers of larvae of the
same age.

For testing, single tomato leaves were placed in a
closed container, with stems hydrated by means of a
small, 3 ml water-filled bottle. Compounds, dissolved
in water or methanol, were sprayed on the tomato
leaves with an ultra fine atomizer. Six, 3-day-old
larvae were placed on each leaf, allowed to feed for
24-108 hours, and then the percentage of leaf remain-
ing was determined by planimetry, weight, or "blind"
visual observation. An active compound was one which
resulted in an increase in percentage of leaf remain-
ing, compared with control.

In some cases, test agents were tested for ova-
cidal activity by dipping groups of 10-50 Manducca
eggs in drug solutions for 60 seconds and then deter-
mining the percentage of eggs which produced viable
larvae. A compound with active ovacidal activity was
one which decreased the percentage of eggs hatched,
relative to control.

EXAMPLE 1
Phenylethylamines as Octopamine Agonists

Table 1 shows the structure/activity relationships
of phenylethylamines interacting with octopamine
sensitive adenylate cyclase of the firefly (Photinus
pyralis).

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TABLE 1

Structure-activity Relationships of Phenylethylamines
Interacting with Octopamine-sensitive Adenylate Cyclase
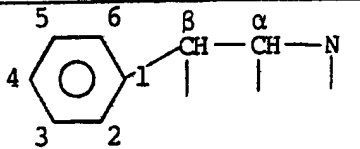
Compound	ring	β CH	α CH	N	Vmax (%OCT)	$\dfrac{K_a OCT}{K_a drug}$
β-phenylethylamine	–	H	H	H_2	13±2	0.03
(+)-amphetamine	–	H	CH_3	H_2	N.A.	–
(±)-phenylethanolamine (-hydroxyphenyl ethylamine	–	OH	H	H_2	45±1	0.11
(±)-norephedrine (phenylpropanolamine)	–	OH	CH_3	H_2	8±1	<0.09
(±)-o-octopamine	2-OH	OH	H	H_2	23±1	0.05
(+)-m-octopamine (norphenylephrine)	3-OH	OH	H	H_2	9±1	0.1
(−)-phenylephrine	3-OH	OH	H	HCH_3	17±1	<0.2
tyramine	4-OH	H	H	H_2	57±1	0.15
(±)-p-hydroxyamphet-amine	4-OH	H	CH_3	H_2	8±3	<0.05
(+)-p-octopamine	4-OH	OH	H	H_2	81±1	0.06
(±)-p-octopamine	4-OH	OH	H	H_2	100±1	1.0
(−)-p-octopamine (natural)	4-OH	OH	H	H_2	101±2	1.3
(+)-N-methyloctopamine (synephrine)	4-OH	OH	H	HCH_3	108±10	1.8
(±)-α-methyloctopamine (p-hydroxynorephedrine)	4-OH	OH	CH_3	H_2	44±2	0.05

TABLE 1 - continued
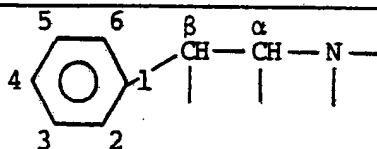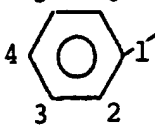
Compound	^{5} ^{6} β α CH—CH—N— 4 1 3 2				Vmax (%OCT)	$\dfrac{K_a OCT}{K_a drug}$
(±)-N,N-dimethyl-octopamine	4-OH	OH	H	(CH$_3$)$_2$	43±4	0.9
(±)-α-methylsynephrine (p-hydroxyephedrine)	4-OH	OH	CH$_3$	HCH$_3$	7±2	<0.01
p-hydroxymandelic acid	4-OH	OH	OHO	–	N.A.*	–
isoxsuprine	4-OH	OH	CH$_3$	1**	N.A.	–
p-methoxphenylethyl-amine (p-methoxytyramine)	4-OCH$_3$	H	H	H$_2$	6±3	0.10
p-fluoro-phenyl-ethanolamine	4-F	OH	H$_2$	H$_2$	72±8	0.11
2,4-dichlorophenyl-ethanolamine	2-Cl,4-Cl	OH	H	H$_2$	14±1	0.67
m-chloro-octopamine	3-Cl,4-OH	OH	H	H$_2$	77±1	0.28
dopamine	3-OH,4-OH	H	H	H$_2$	4±1	0.26
N-methyldopamine	3-OH,4-OH	H	H	HCH$_3$	11±1	0.19
(-)-nonrepinephrine	3-OH,4-OH	OH	H	H$_2$	72±1	0.13
(-)-epinephrine	3-OH,4-OH	OH	H	HCH$_3$	75±2	0.20
(-)-isoproterenol	3-OH,4-OH	OH	H	HCH(CH$_3$)$_2$	19±1	<0.03
(±)-normetanephrine	3-OCH$_3$, 4-OH	OH	H	H$_2$	68±2	0.07

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<u>TABLE 1 - continued</u>

Compound	$\overset{5}{\underset{3}{\overset{6}{\underset{2}{\bigcirc}}}} \overset{\beta}{\underset{|}{CH}} - \overset{\alpha}{\underset{|}{CH}} - \overset{}{\underset{|}{N}} -$				Vmax (%OCT)	$\dfrac{K_a OCT}{K_a drug}$
salbutamol	3-CH$_2$-OH, 4-OH	OH	H	HC(CH$_3$)$_3$	N.A.	-
zinterol	3-NHSO$_2$CH$_3$, 4-OH	OH	H	2**	N.A.	-

*
 No Activity

** 1 $\underset{\overset{|}{CH_3}}{CH}-CH_2O-\bigcirc$

  i.e., $\underset{\overset{||}{O}}{-C-OH}$

**2 $\underset{\overset{|}{CH_3}}{\overset{\overset{CH_3}{|}}{-C}}-CH_2-CH_2-\bigcirc$
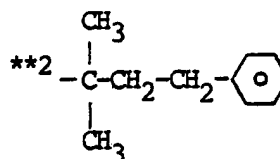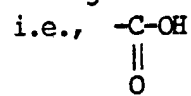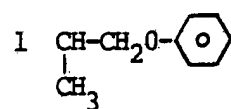
-32-
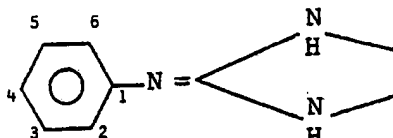
It can be seen that in most instances the half-maximal activation constant K_a for the desired pest controlling compound ranges between less than 0.01 of (±)-p-octopamine to greater than (±)-p-octopamine, whereas the Vmax ranges from less than 4 to 108% of Vmax of octopamine. Examples of compounds satisfying the criteria of most preferred octopamine agonists ($K_a^{oct}/Ka^B>0.1$; Vmax>10%) are p-octopamine, N-methyl-octopamine, p-fluoro-phenylethanolamine, and 2,4-dichlorophenylethanolamine. Note here that m-octopamine has much less activity than the active positional isomer, p-octopamine. As will be shown below, and confirming the _in vitro_/_in vivo_ correlation m-octopamine also has much less pesticidal activity than p-octopamine, and the activity shows much less synergism.

EXAMPLE 2

Clonidines as Octopamine Agonists

A number of clonidine analogues were investigated as octopamine agonists in the octopamine receptor of the firefly lantern. Inhibitory properties were also tested. Table 2 shows the results of these experiments.

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TABLE 2

OCTOPAMINE RECEPTOR ACTIVITY OF THE CLONIDINES

		Vmax (% OCT)	$\dfrac{K_a OCT}{K_a drug}$	K_i (µm)
NC 10	–	N.A.	N.A.	190
NC 12	4-Br	34	3.0	–
NC 8	2-Cl, 4-Cl	47	3.7	–
NC 7	2-CH_3, 4-Cl	68	9.8	–
NC 9	2-CH_3, 4-CH_3	78	5.1	–
NC 2	2-Cl, 5-Cl	9	1.1	23
NC 6	2-Br, 6-Br	35	2.1	–
clonidine	2-Cl, 6-Cl	35	0.95	20
NC 4	2-CH_3, 6-CH_3	80	0.41	–
NC 5	2-CH_2CH_3, 6-CH_2CH_3	97	19.0	–
NC 3	2-Cl, 4-Cl, 5-Cl	80	2.8	–
NC 11	2-Cl, 4-Cl, 6-Cl	60	1.9	–·
NC 13	2-CH_3, 4-CH_3, 6-CH_3	112	4.3	–
NC 14	2-Cl, 4NH_2, 6-Cl	29	0.7	440
NC 15	2-Cl, 4-$N(CH_3)_2$, 6-Cl	38	1.9	–
NC 16	2-Cl, 4-$NCH_3(CH_2CH_2Cl)$, 6-Cl	6	4.8	10
NC 17	2-Cl, 4-$CH_2NCH_3(CH_2CH_2Cl)$, 6-Cl	N.A.	N.A.	250

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Among the findings resulting from this data was
the fact that several clonidine derivatives (some
having low mammalian potency) are extremely potent
octopamine agonists. For example, 2-(2,6-diethyl-
phenylimino) imidazolidine (NC5) was an extremely
potent activator of light organ adenylate cyclase.
Significant stimulation occurred at concentrations as
low as 3×10^{-8}. The K_a was about $1\,\mu M$ (20-fold lower
than that due to octopamine), and maximal stimulation
was equal to that caused by octopamine. In experiments
employing combinations of octopamine and NC5, it was
found that stimulation by NC5 was non-additive with
that due to octopamine, and that NC5 had no activity
as an octopamine antagonist. It was also found that
stimulation of enzyme activity by NC5 was reversible
and could be inhibited by low concentrations of cer-
tain antagonists, such as cyproheptadine, which are
known to inhibit stimulation by octopamine.

Similar to octopamine, NC5 showed a high degree of
selectivity for tissues known to contain an octopa-
mine-activated adenylate cyclase as compared with
those containing dopamine- or adrenergic-stimulated
adenylate cyclases. Thus, isoproterenol potently
stimulates $Beta_1$ and $Beta_2$-adrenergic-stimulated
adenylate cyclase in rat heart and liver. In the
heart, octopamine caused only a small stimulation (16%
that of isoproterenol) while NC5 was almost completely
inactive. In the liver, neither octopamine nor NC5
were active. Similarly, in the rat caudate nucleus,
although dopamine stimulated the dopamine-sensitive
adenylate cyclase known to be present, neither
octopamine nor NC5 caused any significant enzyme

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stimulation. In none of the three mammalian tissues did NC5 cause any inhibition of basal adenylate cyclase activity. Furthermore, other data indicated that the effects of the phenylimino-imidazolidines on octopamine-sensitive adenylate cyclase could be clearly distinguished from their effects on alpha-adrenergic receptors.

Other potent derivatives included: 2-methyl, 4-chloro (NC7) (potency ratio = 9.8); 2,4-dimethyl (ratio = 5.1); 2,4,6- trimethyl (ratio =4.3); 2,4-dichloro (ratio = 3.7); and 2,4,5- trichloro (ratio = 3.0). In prior physiological studies, it had been reported that clonidine, a 2,6-dichloro phenylimino-imidazolidine derivative, can stimulate octopamine receptors which may be distinct from those associated with adenylate cyclase (Evans, P.D., _J. Physiol._ 318: 99-122 (1981)). In the present studies, clonidine was much less potent than the above compounds (octopamine potency ratio $\doteq$ 0.9). In addition, it was less effective (V_{max}=35% that of octopamine), and was a partial antagonist of octopamine-stimulated enzyme activity (K_i = 20 µM).

To confirm that activation of octopamine-sensitive adenylate cyclase by the highly active phenylimino-imidazolidines was indicative of _in vivo_ octopaminergic potency, some of the compounds were evaluated for their ability to stimulate light production when injected into the transected, isolated tails of _Photinus_ males. The dose-response curves showed that, for a group of animals, octopamine stimulated light emission with an EC_{50} for octopamine of about 5 nmoles/tail. NC5, in contrast, had an EC_{50} of about

0.3 nmoles, and thus was more than 16 times as potent
as octopamine. Relative to octopamine, NC5 was even
more potent at low levels of stimulated light emis-
sion. At high doses, maximal stimulation by the two
compounds was comparable and, at maximally effective
doses, a combination of both compounds did not elicit
more light production than either compound alone.

In order to confirm that the phenylimino-imida-
zolidines have anti-feeding behavioral actions, the
effects of certain of these compounds, as well as
octopamine, itself, were examined on the feeding
behavior of Manducca sexta (tobacco hornworm) larvae.
Application of octopamine to leaves resulted in a
dose-dependent inhibition (K_i 0.5 mg/leaf) of feeding
by the larvae. 2-(2-methyl, -4-chlorophenylimino)
imidazolidine (NC7) caused qualitatively similar
effects but was over 15-fold more potent than octopa-
mine (K_i 0.01 mg/leaf). Larvae feeding on leaves
treated with both compounds showed tremors, hyper-
activity, and leaf walk-off behavior. At higher
doses, larvae demonstrated particular difficulty
during molting (a task demanding of motor coordina-
tion), frequently being unable to shed their old
cuticle, and subsequently dying. Together, the in
vivo studies confirm the in vitro screen, indicating
that in vitro selected phenylimino-imidazolidines are
very potent agonists of octopamine-sensitive cyclase.

EXAMPLE 3

Demonstration of Octopamine Agonist
Activity Measured in Tissue from an Insect Pest

In order to show that adenylate cyclase activation
in tissue from an insect pest can also be used to
define octopamine agonists, members of three chemical

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groups   (phenylethanolamines,   phenyliminoimidazoli-
dines, and formamidines) were tested for their ability
to activate adenylate cyclase in broken cell prepara-
tions of insect nerve cord.   Figure 1 shows that
octopamine, NC7 (see Table 2), and DDCDM were potent
activators of adenylate cyclase in nerve cord of
tobacco hornworm.   Octopamine and NC7 fulfilled the
criteria of being most preferred octopamine agonists.
As will be described below, these compounds have
pesticidal activity.

EXAMPLE 4

A Direct Stimulator of Adenylate Cyclase
in the Firefly Lantern and in Pest Tissue

Fig. 2 shows the effect of forskolin in directly
activating adenylate cyclase in firefly lantern.
Figure 3 also shows the effect of forskolin in direct-
ly activating adenylate cyclase activity in broken
cell perparations from the nerve cord of tobacco horn-
worm larvae.   In both tissues, forskolin fulfills the
criteria of a preferred enzyme activator.   As will be
shown below, forskolin has activity as a pesticide and
this activity is synergized by phosphodiesterase
inhibition.

EXAMPLE 5

Demonstration of Increased Cyclic AMP Content in
Pest Nerve Cord Caused by a Combination of an
Octopamine Agonist and a Phosphodiesterase Inhibitor

As described earlier and as shown in Scheme I, the
pesticidal activity of octopamine agonists is mediated
through the increased formation of cyclic AMP within
the cells of the insect pest.   In order to directly
demonstrate that a PDE inhibitor, as defined above,
can augment the increase in cyclic AMP caused by an

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octopamine agonist (as defined above) in an insect
pest, further experiments were run using intact nerve
cords of tobacco hornworms. These intact nerve cords
were incubated under physiological conditions in the
presence of octopamine, NC7, or DDCDM, first in the
absence of PDE inhibitor IBMX and then in the presence
of 0.1 mM IBMX. After 5 minutes, the intact nerve
cord was quickly treated to release all cyclic AMP
which was present within the tissue.

Table 3 compares the levels of cyclic AMP within
nerve tissue under different treatments. As can be
seen, with all three octopamine agonists (octopamine,
NC7, and DDCDM), addition of IBMX markedly increased
the level of cyclic AMP within the nerve tissue.

TABLE 3

Effect of Octopamine Agonists and IBMX on
Increasing Cyclic AMP in Insect Pest Tissue

Compound	Cyclic AMP Content (Fold-increase over Control)	
	$-$IBMX	$+0.1$ mM IBMX
Octopamine	0.9 ± 0.3	6.4 ± 1.0
NC 7	2.2 ± 0.2	13.9 ± 3.0
DDCDM	5.4 ± 3.8	11.1 ± 2.7

Also of considerable interest was the fact that,
consistent with the invention, the level of cyclic AMP
within the tissue paralleled pesticidal activity.
Thus, in other experiments, it was determined that the
rank order antifeeding effectiveness of the various
compositions (based on the EC_{50} of the octopamine
agonist) shown was: DDCDM+IBMX = NC7+IBMX > Octopa-
mine+IBMX = DDCDM alone > NC7 alone > octopamine

alone. This order was identical to the rank order of
the cyclic AMP levels shown in Table 3. Also note,
for example, that, in the absence of IBMX, octopamine
caused little elevation of cyclic AMP, and likewise
had little pesticidal activity (see Figs. 9, 10
below). In the presence of IBMX, however, octopamine
considerably elevated both cyclic AMP content and
pesticidal activity.

EXAMPLE 6

In Vivo Leaf Test of Compositions
Containing Phenylethanolamines

Table 4 shows the results obtained for mixtures
comprising various phenylethanolamines in the presence
or absence of IBMX as a PDE inhibitor.

TABLE 4

Agonist (gm/100ml)		% Leaf Remaining	
		No PDE Inhibitor	PDE Inhibitor (0.1 g/100 ml)
4-F-phenylethanol-amine	C*	<5	IBMX 25
	10^{-3}	<5	50
	10^{-2}	<5	80
	10^{-1}	<5	98
	0.3	25	95
2,4-dichlorophenyl-ethanolamine	C*	<5	IBMX 20
	10^{-2}	<5	30
	10^{-1}	<5	50
	1	10	80
Synephrine	C*	<5	IBMX 42
	10^{-2}	<5	42
	10^{-1}	<5	55
	1	30	90

*Control = No Agonist

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The results show that the phenylethanolamines
themselves had antifeeding activity and that, in all
instances, addition of IBMX markedly enhanced the
ability of the phenylethanolamines to inhibit the leaf
feeding activity of the pest.

EXAMPLE 7
In Vivo Leaf Tests of Compositions
Containing Phenyliminoimidazolidines (Clonidines)

Mixtures of various clonidine derivatives with or
without IBMX were prepared and tested in the
aforementioned in vivo tests.   Table 5 shows the
results.

TABLE 5

Agonist (gm/100ml)		% Leaf Remaining	
		No PDE Inhibitor	PDE Inhibitor (0.1 g/100 ml)
NC 7[1]	C*	25	IBMX 30
	10^{-3}	23	48
	10^{-2}	30	85
	10^{-1}	55	95
	3×10^{-1}	80	100
clonidine	C*	5	IBMX 35
	10^{-2}	5	55
	10^{-1}	20	55
	1	65	63
NC 5[1]	C*	10	IBMX 18
	10^{-3}	10	17
	10^{-2}	15	30
	10^{-1}	30	45
	3×10^{-1}	40	70
NC 10[1]	C*	10	IBMX 15
	10^{-3}	5	12
	10^{-2}	5	15
	10^{-1}	5	13
	1	5	15

(1) See Table 2, supra

*Control = No agonist

The data indicate that, with NC7, clonidine, and NC5, addition of IBMX results in substantial increases in the potency of the pest controlling compound. It will be noted that these three pest controlling compounds, on the basis of in vitro testing (Table 2), all satisfied the criteria of being most preferred compounds of type 1. Of interest, and further confirming the invention, was the fact that the structural analogue, NC10, was inactive in vivo. Thus, as can be seen in Table 5, NC10 lacked antifeeding

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activity both in the absence or presence of the PDE
inhibitor, IBMX. This unexpected result was correctly
predicted by the in vitro assay data shown in Table 2,
where NC10 had no activity.

EXAMPLE 8
In Vivo Leaf Test of a Composition
Containing a Direct Enzyme Stimulator.

As described previously, the diterpene forskolin
is an example of a compound which directly stimulates
adenylate cyclase, thereby producing cyclic AMP. Fig.
4 shows that forskolin, alone, has antifeeding acti-
vity. Fig. 4 also shows that this antifeeding
activity is enhanced in the presence of (0.1%) of the
PDE inhibitor IBMX.

EXAMPLE 9
In Vivo Leaf Test of a Composition
Containing a cAMP Analogue

Two cyclic AMP analogues were tested by the leaf
test. The results are shown in Figure 5 for
p-Cl-phenylthio cyclic AMP, and Figure 6 for n-butyl-
aminobenzylthio cyclic AMP. As can be seen, both
compounds, at a concentration of 1%, showed antifeed-
ing activity in the absence of IBMX. The activity of
both compounds was increased by addition of IBMX.

It has been previously shown, above, that PDE
inhibition can increase the concentration of cyclic
AMP in insect tissue. To further confirm the inven-
tion this example demonstrates directly that PDE
inhibition can also increase the levels of cyclic AMP
analogues, such as butyl-benzylthio cyclic AMP, in
insect pest tissue. In order to do this, a fixed
amount of butyl-benzylthio cyclic AMP was incubated in
vitro for 4 hours in the presence of a tissue homoge-
nate from tobacco hornworm nerve cord. In some tubes,

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IBMX (0.1 mM) was added to inhibit PDE. After the incubation, the amount of butyl-benzylthio cyclic AMP remaining was measured by protein binding assay (Brown et al., supra), using a standard curve based upon butyl-benzylthio cyclic AMP. Fig. 7 shows that addition of IBMX more than doubled the amount of butyl-benzylthio cyclic AMP present after 4 hours.

Having now fully described this invention it will be understood by those of skill in the art that the same can be performed within a wide and equivalent range of compositions, parameters, structures, modes of application, pests, formulations, and ranges without effecting the scope of the invention or or any embodiment thereof.

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CLAIMS

1. A method of controlling a pest which comprises bringing into contact with said pest a pest-controlling amount of an agent selected from the group consisting of

   (1) a non-formamidine -p-octopamine agonist having substantial activity toward an octopamine receptor present in said pest;

   (2) a compound directly and substantially stimulating the enzyme, adenylate cyclase; and

   (3) a cyclic adenosine monophosphate (cAMP) analogue.

2. The method of claim 1 wherein said agent is a non-formamidine octopamine agonist toward an octopamine receptor present in said pest.

3. The method of claim 1 wherein said agent directly stimulates the enzyme adenylate cyclase.

4. The method of claim 1 wherein said agent is a cAMP analogue.

5. The method of claim 2 wherein the _in vitro_ ratio $Ka^{oct}/Ka^{(1)}$ for said compound is greater that 0.05; where Ka^{oct} is the agonistic activity constant for $(\pm)$p-octopamine

6. The composition of claim 5 wherein said ratio is between 0.05 and 1000.

7. The composition of claim 6 wherein said ratio is between 0.1 and 1000.

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8. The composition of any of claims 2, 5, 6 or 7 wherein the _in vitro_ % Vmax for said compound, relative to the Vmax for ($\pm$) p-octopamine, is greater than 5%.

9. The method of claim 8 wherein said % Vmax is between 10 and upwards of 100%.

10. The composition of claim 2 wherein the _in vitro_ ratio $Ka^{oct}/Ka^{(1)}$ for said agent (1) is greater than 0.1 and the % Vmax for said compound, relative to the Vmax for ($\pm$)-p-octopamine is greater than 10%

11. The method of claim 3 wherein, at a concentration less than 1 mM said agent is capable of causing _in vitro_ stimulation of said enzyme of at least 10% that due to a Vmax concentration of ($\pm$)-p-octopamine.

12. The method of claim 4 wherein said agent has a $Ka^{(3)}/Ka^{cAMP}$ ratio greater than 0.01, wherein Ka^{cAMP} is the activation constant for cAMP.

13. The method of claim 12 wherein said ratio is between 0.05 and 100 or more.

14. The method of any of claims 4, 12 or 13 wherein the Vmax for said compound is at least 5% of the Vmax for cAMP.

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15. The method of claim 1 wherein said agent is
in a composition in the form of a dust, a powder, a
water dispersion, an emulsion or a solution.

16. The method of claim 1 wherein said agent is
present in composition together with a pesticidal
carrier.

17. The method of claim 2 wherein said agent has
formula

$$R^{10} \underset{\underset{R^{12}}{R^{11}}}{\bigcirc} - \underset{\underset{R^6}{|}}{CH} - \underset{\underset{R^7}{|}}{CH} - N \overset{R^8}{\underset{R^9}{\diagdown}}$$
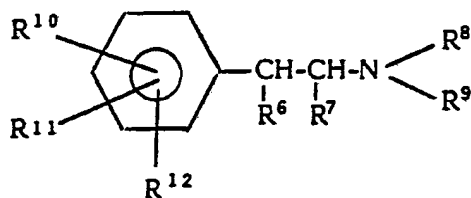
where R^6 and R^7 are the same or different and
selected from the group consisting of H, OH and lower
alkyl.

R^{10}, R^{11} and R^{12} are the same or different
and selected from the group consisting of hydrogen,
hydroxy, fluorine, chlorine, bromine, iodine, nitro,
lower (C_1-C_6) alkyl, lower (C_1-C_6) alkoxy, lower halo-
alkyl, amino, mono lower alkylamino, di-lower alkyl
amino, hydroxy substituted lower alkyl and lower
acylamino.

R^8 and R^9 are H, lower alkyl, or lower alkyl
substituted by hydroxy or lower alkoxy

18. The method of claim 2 wherein said agent (1)
has the formula
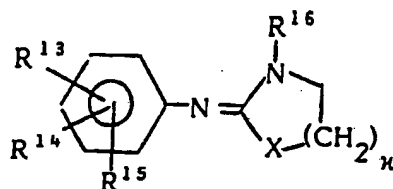
where R^{13}, R^{14}, R^{15} are the same or different and
selected from the group consisting of hydrogen,
hydroxy, fluorine, chlorine, bromine, iodine, nitro,
lower (C_1-C_6) alkyl, lower (C_1-C_6) alkoxy, lower halo-
alkyl, amino, mono lower alkylamino, di-lower alkyl
amino, hydroxy substituted lower alkyl and lower
acylamino;

    R^{16} is H, lower alkyl or lower alkyl sub-
stituted by hydroxy or lower alkoxy;

    X is O, CH2, S or NR ^{16};

    n is 1 or 2;

    or R^{13}, R^{14} or R^{13}, R^{15} taken together form
a phenyl or cyclohexyl fused to the phenyl ring.

19. The method of claim 2 wherein said agent (1)
has formula
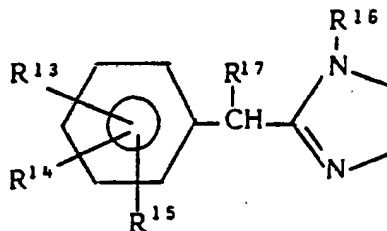
where R^{13}, R^{14} and R^{15} are the same or different and selected from the group consisting of hydrogen, hydroxy, fluorine, chlorine, bromine, iodine, nitro, lower (C_1-C_6) alkyl, lower (C_1-C_6) alkoxy, lower haloalkyl, amino, mono lower alkylamino, di-lower alkyl amino, hydroxy substituted lower alkyl and lower acylamino;

R^{16} is H, lower alkyl or lower alkyl substituted by hydroxy or lower alkoxy; R^{17} is H or OH;

or R^{13}, R^{14} or R^{13}, R^{15} taken together form a phenyl or cyclohexyl fused to the phenyl ring.

20. A pest-controlling composition in the form of a dust comprising a pest controlling amount of an agent selected from the group consisting of

(1) a non-formamidine octopamine agonist having substantial activity toward an octopamine receptor present in said pest;

(2) a compound directly and substantially stimulating the enzyme adenylate cyclase; and

(3) a cyclic adenosine monophosphate (cAMP) analogue; together with a pesticidally inert dust carrier.

21. The composition of claim 20 wherein said carrier is selected from the group consisting of organic flour, sulfur, silicon oxides, lime, gypsum, talc, pyrophyllite, bentonite, kaolins, attapulgite, and volcanic ash.

22. A pest controlling composition in the form of a granule or pellet comprising a pest controlling

amount of an agent selected from the group consisting
of

(1)   a non-formamidine octopamine agonist having
      substantial activity toward an octopamine
      receptor present in said pest;

(2)   a compound directly and substantially stimu-
      lating the enzyme adenylate cyclase; and

(3)   a cyclic adenosine monophosphate (cAMP)
      analogue, together with a granule or pellet
      forming inert carrier.

23. The composition of claim 22 wherein said
granule or pellet forming carrier is selected from the
group consisting of organic flour, sulfur, silicon
oxides, lime, gypsum, talc, pyrophyllite, bentonites,
kaolin, attapulgite, and volcanic ash.

24. A pest controlling composition in the form of
an aerosol which comprises a pest controlling amount
of an agent selected from the group consisting of

(1)   a non-formamidine octopamine agonist having
      substantial activity toward an octopamine
      receptor present in said pest;

(2)   a compound directly and substantially stimu-
      lating the enzyme adenylate cyclase; and

(3)   a cyclic adenosine monophosphate (cAMP)
      analogue, together with atomizing amount of a
      liquefied gas.

25. The composition of any of claims 20-24
wherein said agent is a non-formamidine octopamine
agonist (1) toward an octopamine receptor present in
said pest.

26. The composition of claim 25 wherein the <u>in vitro</u> ratio $K_a^{oct}/K_a^{(1)}$ for said compound (1) is greater than 0.05.

27. The composition of claim 26 wherein said ratio is between 0.05 and 1000.

where R^{13}, R^{14} and R^{15} are the same or different and selected from the group consisting of hydrogen, hydroxy, fluorine, chlorine, bromine, iodine, nitro, lower (C_1-C_6) alkyl, lower (C_1-C_6) alkoxy, lower halo-alkyl, amino, mono lower alkylamino, di-lower alkyl amino, hydroxy substituted lower alkyl and lower acy-lamino;

R^{16} is H, lower alkyl or lower alkyl sub-stituted by hydroxy or lower alkoxy; R^{17} is H or OH;

or R^{13}, R^{14} or R^{13}, R^{15} taken together form a phenyl or cyclohexyl fused to the phenyl ring.

(cancelled)   20. A pest-controlling composition in the form of a dust comprising a pest controlling amount of an agent selected from the group consisting of

  (1)   a non-formamidine octopamine agonist having substantial activity toward an octopamine receptor present in said pest;

  (2)   a compound directly and substantially stimu-lating the enzyme adenylate cyclase; and

  (3)   a cyclic adenosine monophosphate (cAMP) ana-logue; together with a pesticidally inert dust carrier.

(cancelled)   21. The composition of claim 20 wherein said carrier is selected from the group con-sisting of organic flour, sulfur, silicon oxides, lime, gypsum, talc, pyrophyllite, bentonite, kaolins, attapulgite, and volcanic ash.

(cancelled)   22. A pest controlling composition
in the form of a granule or pellet comprising a pest
controlling amount of an agent selected from the group
consisting of

    (1)   a non-formamidine octopamine agonist having
        substantial activity toward an octopamine
        receptor present in said pest;

    (2)   a compound directly and substantially stimu-
        lating the enzyme adenylate cyclase; and

    (3)   a cyclic adenosine monophosphate (cAMP) ana-
        logue, together with a granule or pellet
        forming inert carrier.

(cancelled)   23. The composition of claim 22
wherein said granule or pellet forming carrier is
selected from the group consisting of organic flour,
sulfur, silicon oxides, lime, gypsum, talc, pyrophyl-
lite, bentonites, kaolin, attapulgite, and volcanic
ash.

(cancelled)   24. A pest controlling composition
in the form of an aerosol which comprises a pest con-
trolling amount of an agent selected from the group
consisting of

    (1)   a non-formamidine octopamine agonist having
        substantial activity toward an octopamine
        receptor present in said pest;

    (2)   a compound directly and substantially stimu-
        lating the enzyme adenylate cyclase; and

    (3)   a cyclic adenosine monophosphate (cAMP) ana-
        logue, together with atomizing amount of a
        liquefied gas.

(cancelled)   25. The composition of any of
claims 20-24 wherein said agent is a non-formamidine
octopamine agonist (1) toward an octopamine receptor
present in said pest.

(cancelled)   26. The composition of claim 25
wherein the $\underline{in}$ $\underline{vitro}$ ratio $K_a^{oct}/K_a^{(1)}$ for said com-
pound (1) is greater than 0.05.

(cancelled)   27. The composition of claim 26
wherein said ratio is between 0.05 and 1000.

28. (new) The method of claim 3 wherein said com-
pound is selected from the group consisting of diter-
penes and forskolin and derivatives thereof repre-
sented by the formula:
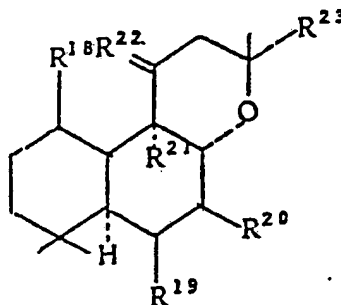
wherein R^{18-23} stands for hydrogen, hydroxy, oxy,
keto, lower alkyl, lower alkene, lower alkoxy, carboxy
and carboxy-amino; and,
wherein in forskolin, $R^{18}=R^{19}=R^{21}=OH$; $R^{20}=OCOMe$;
$R^{22}=O$; and $R^{23}=CH=CH_2$.

29. (new) The method of claim 4 wherein said cAMP analogue is selected from the group consisting of 6-n-butylamino-8 benzylthio-cyclic AMP; 8-p-chloro-phenylthio-cyclic AMP; 8-chlorocyclic AMP; 8-bromocyclic AMP; N^6-monobutyryl or N^6, 2'-0-dibutyryl cyclic AMP; 7-deaza-cyclic AMP; and 1-deaza-cyclic AMP.

30. (new) A pest controlling composition comprising an pest-controlling amount of a non-formamidine octopamine agonist having substantial activity toward an octopamine receptor present in a pest and a pesticidally inert carrier.

31. (new) The composition of claim 30 wherein the in vitro ratio $K_a^{oct}/K_a^{(1)}$ for said non-formamidine octopamine agonist is greater than 0.05.

32. (new) The composition of claim 31 wherein said ratio is between 0.05 and 1000. ·

33. (new) The composition of claim 30 which is in the form of a dust.

34. (new) The composition of claim 28 which is in the form of a granule or pellet.   .

35. (new) The composition of claim 33 or 34 wherein said carrier is selected from the group consisting of organic flour, sulfur, silicon oxides, lime, gypsum, talc, pyrophyllite, bentonite, kaolins, attapulgite, and volcanic ash.

36. (new) The composition of claim 28 which is in the form of an aerosol.

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FIG. 1

A. FIREFLY LANTURN   2/5

KaRatio=0.22
Umax(OCT)=100%

FORSKOLIN (μm)

FIG. 2

B. HORNWORM NERVE CORD

KaRatio=0.20
Umax(OCT)=170%

FORSKOLIN (μm)

FIG. 3

FIG. 4

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FIG.6
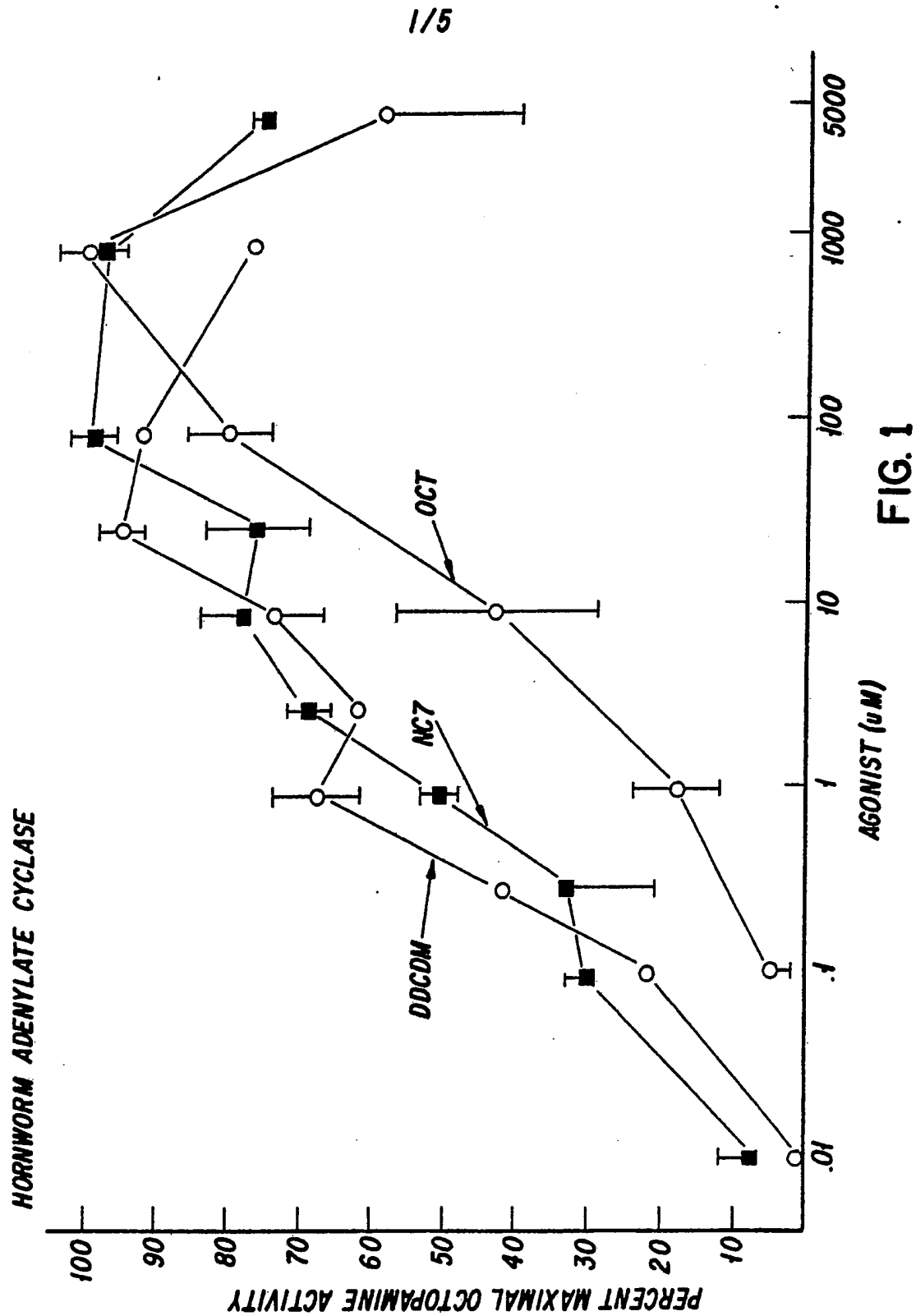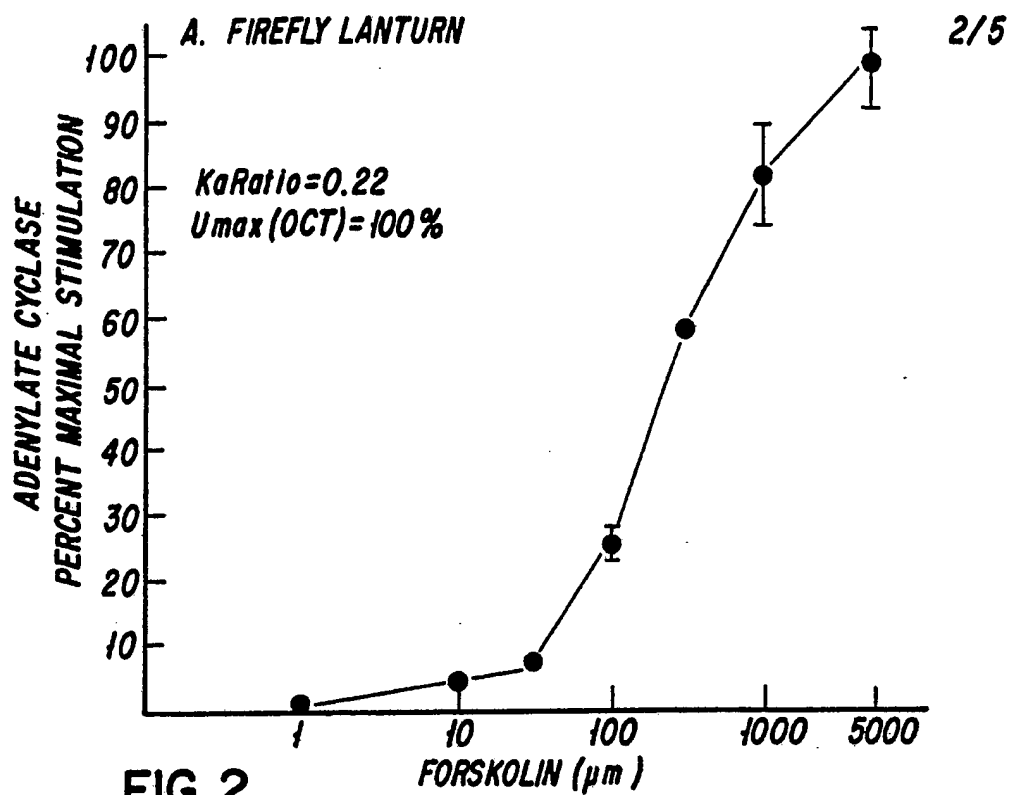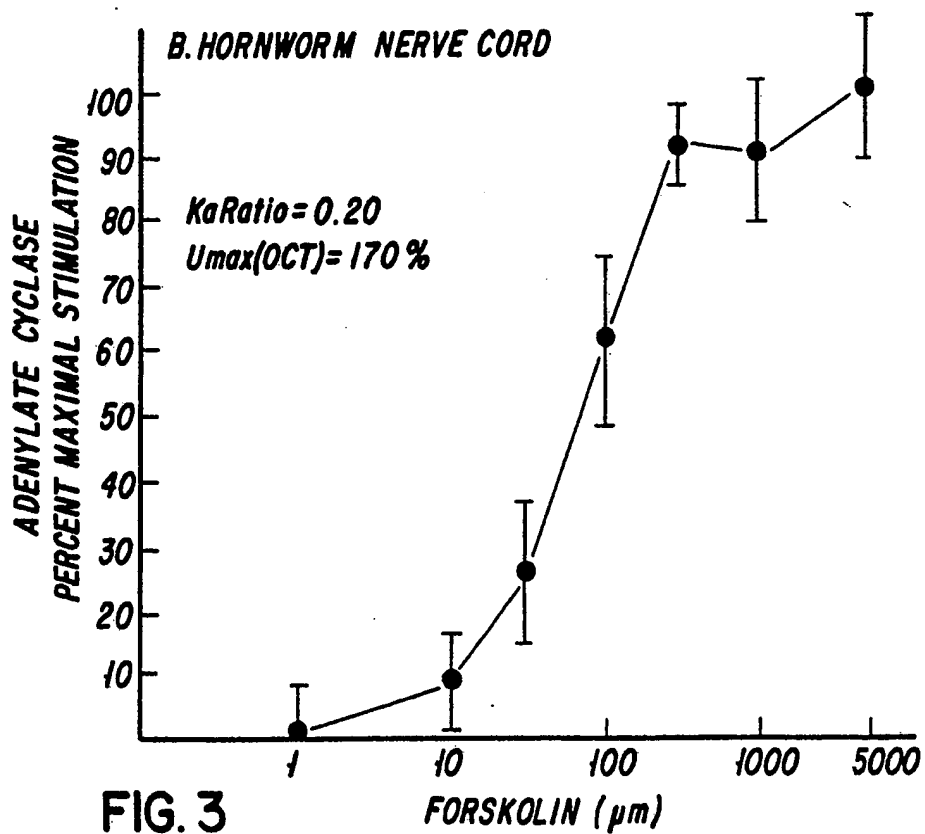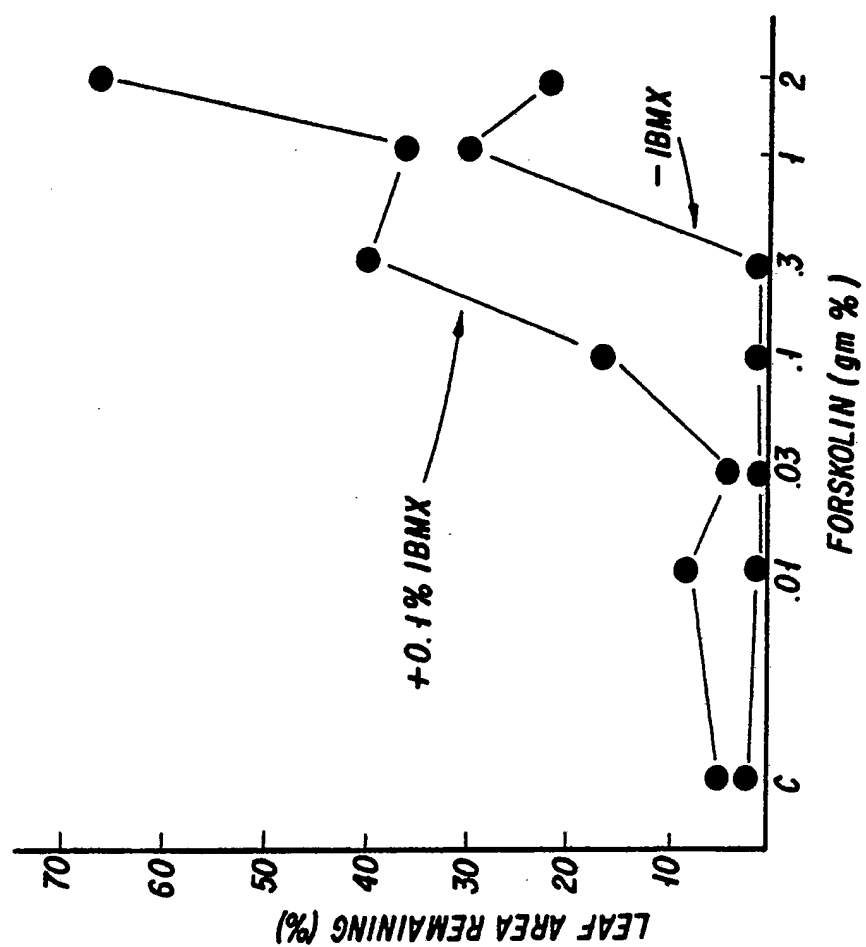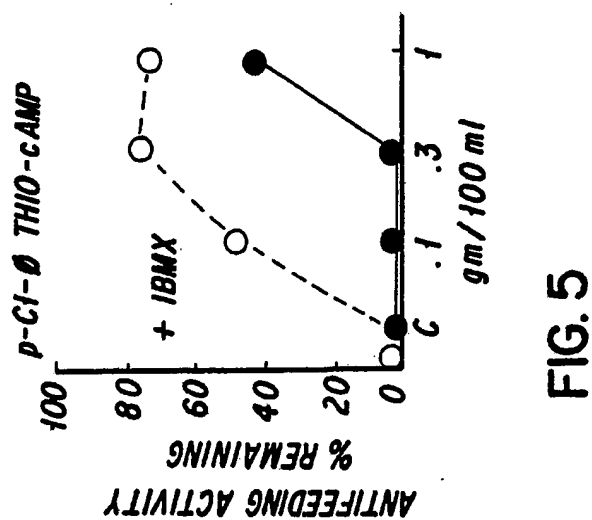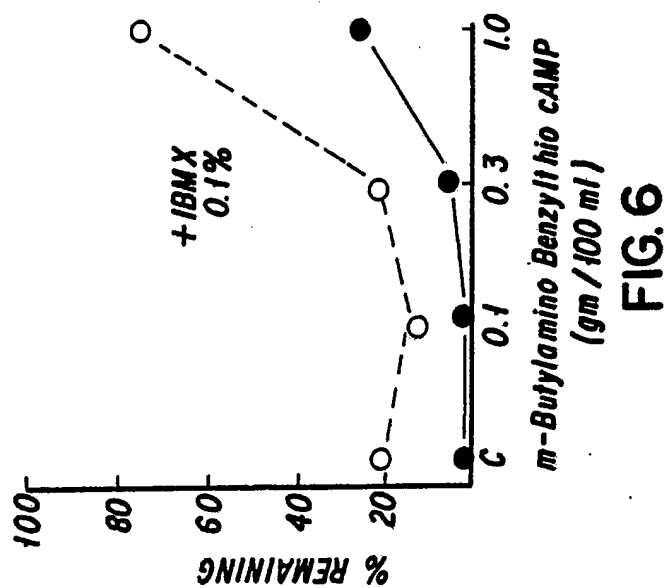
FIG.5

FIG. 7

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁸

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.3 A61L 9/04;A01N 43/90;A01N 43/50;A01N 37/30;A01N 37/52
USCl 414/45; 514/263; 514/396; 514/554; 514/631

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
U.S.	424/45

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	USA, 4,342,585, Published 3 August 1982, Y. Motomura et al	24-27
X	USA, 4,302,457, Published 24 November 1981, J. Voorhees et al.	20-23 & 25-27
X	USA, 4,134,986, Published 16 January 1979, B. Bajwa et al.	20-23 & 25-27
X	USA, 4,118,508, Published 03 October 1978, S. Bhat et al.	20-23, & 25-27
A	R. M. Hollingworth, et al Scientific Papers of the Institute of Organic and Physical Chemistry of The Wroclaw Technical University No. 22, Conference No. 7, 1980.	1-27
A	Science, Volume 208, issued 4 April 1980 (Washington, D.C.) Hollingworth et al., see pages 74-76.	1-27
A	Nature, Volume 287, issued 4 September 1980, (Eng.) P. Evans, see pages 60-62.	1-27

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²
10 July 1985	23 JUL 1985
International Searching Authority ¹	Signature of Authorized Officer ⁴⁰
ISA/US	J.W. Rollins

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	Molecular Pharmacology, Volume 20, issued 1981 (The American Society for Pharmacology and Experimental Therapeutics) J. Nathanson et al., see pages 68-78.	1-27
A	Journal of Argicultural Food Chemistry, Volume 25, No. 3, issued 1977, Chang et al see pages 493-501.	1-27
A	Journal of Medicinal Chemistry Volume 26, No. 3, issued 1983, Seamon et al., see pages 436-439.	1-27
A	Science, Volume 180, issued 4 April 1973, (American Association for the Advancement of Science) Nathanson et al., see pages 308-310.	1-27
A	Since, Volume 203, issued 5 January 1979, (American Association for the Advancement of Science) J. Nathanson, see pages 65-68.	1-27
A	J. Nathanson, in Trace, Amines and The Brain, issued 1976, Usdin et al (editors) see pages 161-190.	1-27